# Insect-host phenological synchrony: foliar terpenes and free amino acids in relation to European pine sawfly life cycle

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## Abstract


- Plants are consumed by some insects only at specific periods of the season.
- Although pine trees hold their foliage all year, the European pine sawfly, a destructive insect pest of pine, feeds only during a narrow "window" of time in early spring.
- We hypothesized that European pine sawfly life cycle is constrained to a narrow temporal window by phenological changes in plant chemistry that result in a limited period of host suitability.
- Consistent with the predictions of this phenological window hypothesis, larval growth and survival decreased as host-insect synchronicity was modified.
- Strong correlations between foliar concentration of free amino acids and insect growth, consumption rate, and efficiency of conversion of ingested food, provide new evidence for their role in the nutritional ecology of the European pine sawfly.


## Introduction

European pine sawfly (*Neodiprion sertifer*) is a destructive defoliator of pine trees (*Pinus* spp.)
(Kolomiets et al. 1972)

**Natural phenological synchrony**


**Eggs hatch as shoots begin to elongate**

**One generation per year**


**Feeding occurs only during a narrow temporal window (mid April to late May)**


**New shoots elongate as pine phenology advances**

**Why do larvae feed only in early spring when mature needles occur all year?**

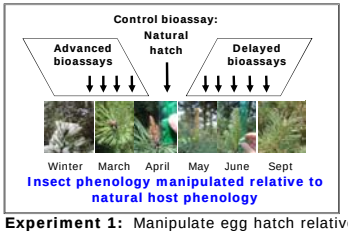
We hypothesized there exists a narrow phenological window of host susceptibility because of ontogenetic changes in host defenses and nutritional quality.

To test this hypothesis, we manipulated insect and host phenology and quantified ontogenic variation in foliar terpenes and free amino acids in relation to insect growth and survival.

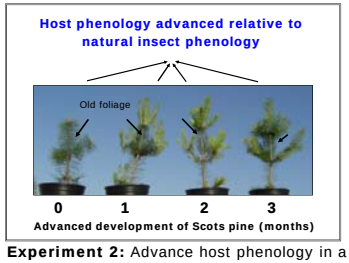
It is expected that concentrations of terpenes (host defenses) increase (Llusià and Peñuelas 2000), and free amino acids (primary nutrients) decrease (Nashölm and Ericsson 1989) as pine phenology advances.

## Methodology

Two experimental approaches to manipulate host/insect phenology: we modified **(1)** insect phenology while maintaining natural host phenology, or **(2)** host phenology while maintaining natural insect phenology:




**Experiment 1:** Manipulate egg hatch relative to host phenology, and repeat feeding assays* over time (before, during, and after natural hatch), measuring changes in insect growth and survival.



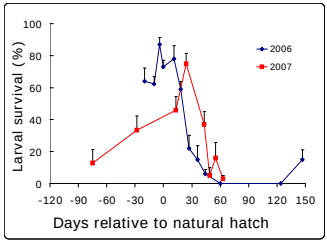

**Experiment 2:** Advance host phenology in a greenhouse by 0 (control), 1, 2, & 3 months relative to natural insect hatch date in mid-April and quantify changes in needle chemistry relative to insect performance.

* **Bioassays:** Groups of 10 neonates were fed mature foliage in the lab (20°C, 15hDL). After 7 days, growth and survival was measured. We used neonates in all assays to avoid stage-dependent confounding effects.

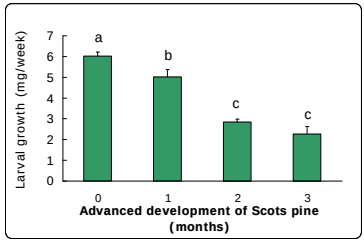
## Results

### Does insect – host phenological synchrony matter? … YES!

Insect survival and growth decreased as insect-host synchrony was manipulated. Pine needles are more susceptible during a narrow "phenological window" of susceptibility to the herbivore. Pine resistance increased as shoots elongate (linear effect: r=0.79, *P* <0.001).




**Experiment 1:** Each year, feeding assays were conducted on previous-year needles (old foliage). In 2007, host quality was highest after the time of natural egg hatch, which greatly decreased survival of early hatching larvae. This density-independent mortality factor may play a role on sawfly population dynamics.

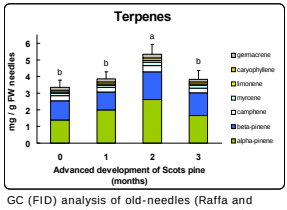



**Experiment 2:** Advancing host phenology relative to natural time of insect egg hatch also had a large effect on European pine sawfly growth, confirming the hypothesis of a phenological window of susceptibility (LSD. $F_{3,36}$=40.31, $P$<0.001).

### How does foliar chemistry changes as pine phenology advances?

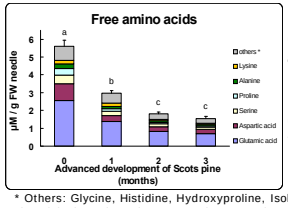
#### I. Plant defensive compounds

- The strength of pine defense did not relax over time.
- Equal or higher level of terpenes as phenology advanced.




GC (FID) analysis of old-needles (Raffa and Steffeck 1988). (LSD. $F_{3,36}$=3.06, $P$=0.04)

#### II. Plant nutritive value

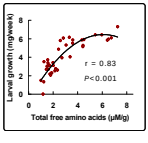


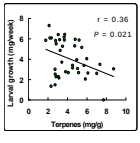

- Concentration of essential free amino acids (not bound to proteins) declined over time.

* Others: Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Phenylalanine, Threonine, Tryptophan, Tyrosine, & Valine.
GC (FID) analysis of old-needles using EZ:Faast kit (Phenomenex). (LSD. $F_{3,36}$=79.37, $P$<0.001)

### How does foliar chemistry relates to insect performance?

**Primary role of free amino acids and secondary role of terpenes in host quality.**

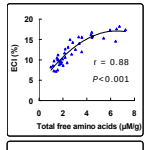


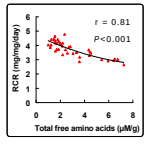

The ratio of free amino acids:terpenes explained less variation (69%) in insect growth than amino acids (83%) alone, suggesting 'nutrient depletion' in old needles is a more important determinant of host quality than plant defenses.

**As amino acid levels declined, host quality declined:**

**Poorer food resource:** Efficiency of Conversion of Ingested food into body mass (ECI) decreased 50%.




**Compensatory feeding:** 31% increase in relative consumption rate (RCR) as amino acids decline partially offsets reduced nutritional quality.




## Conclusions

- **There is a phenological window of host susceptibility to European pine sawfly resulting from ontogenetic changes in needle chemistry.**
- **As host phenology advanced, concentration of free amino acids declined but terpene concentrations (defenses) did not decrease.**
- **As host phenology advanced relative to insect phenology, larval growth and survival declined. Larvae ate more to compensate, but were less efficient converting food to body mass.**
- **These novel findings increase our understanding of the role of ontogenetic variation in host chemistry in the evolution of insect life cycles.**

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